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Supplemental information

Introduction of synaptotagmin 7 promotes facilitation at the climbing fiber to Purkinje cell synapse

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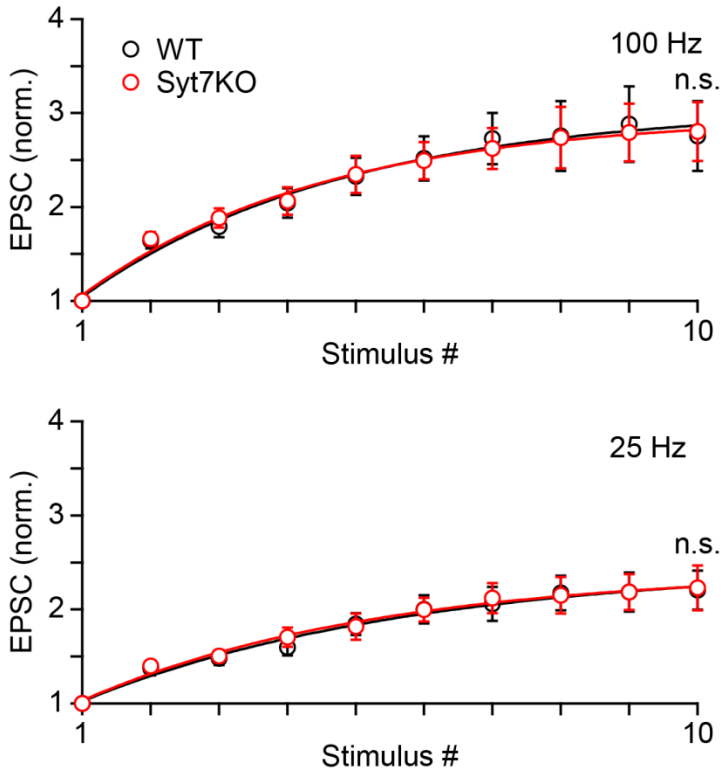

Figure S1: Synaptic train facilitation does not depend on Synaptotagmin 7 at the cerebellar climbing fiber to Purkinje cell (CF-PC) synapse. Related to Figure 1. Experiments were performed in wild-type (WT, black) and Synaptotagmin 7 knockout (Syt7KO) animals at the CF-PC synapse under conditions of low (~0.3 mM) external calcium. The summary for the normalized average EPSC amplitudes is shown for the 100 Hz (*upper*) and 25 Hz (*lower*) trains. Data shown as mean ± SEM.

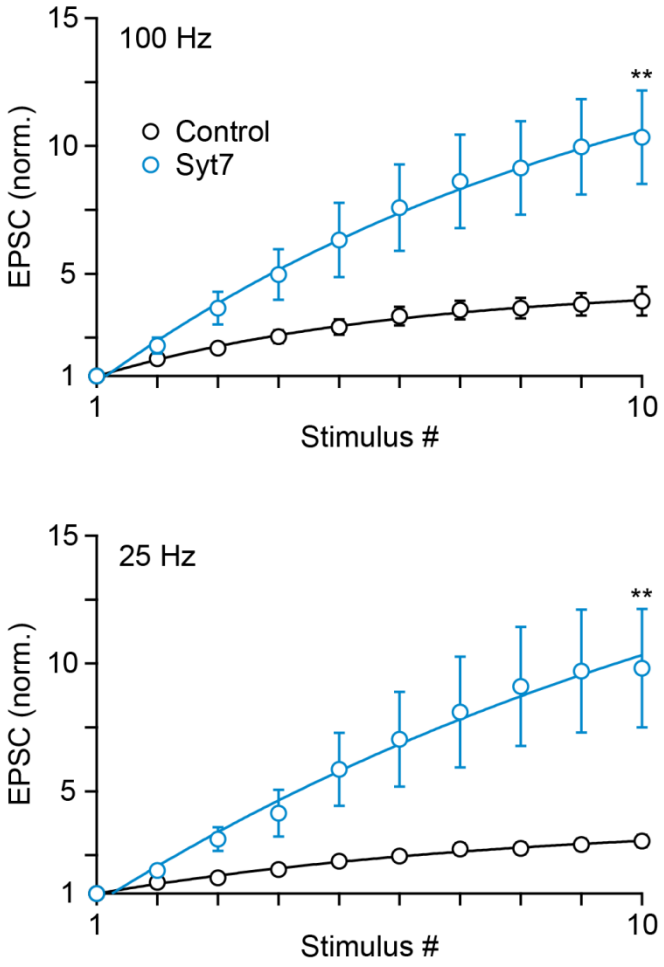

Figure S2: Expression of Syt7 in climbing fibers leads to a drastic increase in train facilitation at climbing fiber to Purkinje cell (CF-PC) synapses. Related to Figure 3. Train facilitation experiments were performed in ChR2 (black) and Syt7-ChR2 (light blue) injected animals at the CF-PC synapse under conditions of low (~0.3 mM) external calcium. The summary for the normalized average EPSC amplitudes is shown for the 100 Hz (*upper*) and 25 Hz (*lower*) train. Statistical significance (unpaired t-test; see Table S1): ** $p<0.01$ Data shown as mean ± SEM.

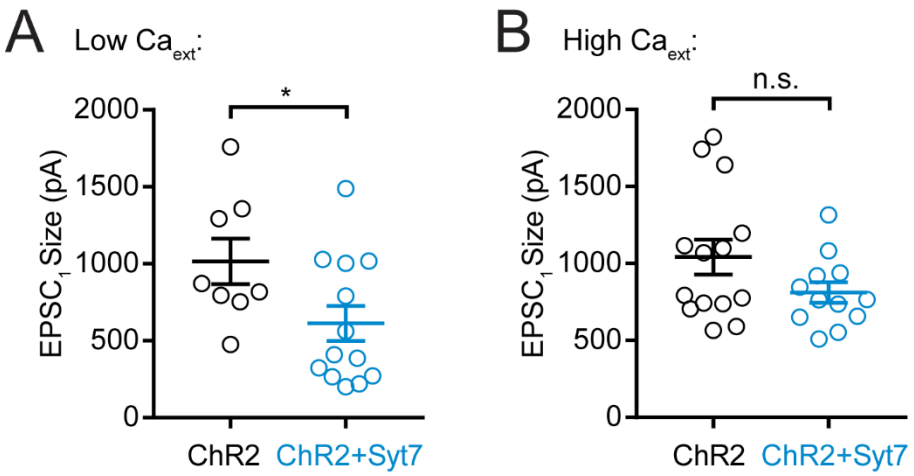

Figure S3: The effect of synaptotagmin 7 expression on EPSC1 size. Related to Figure 3. Experiments were performed in ChR2 (black) and Syt7-ChR2 (light blue) injected animals at the CF-PC synapse under conditions of low (A) or high (B) external calcium.
(A, B) Comparison of EPSC1 sizes across all low (A) and high (B) external calcium PPR experiments. Scatter plots with mean ± SEM indicated. Statistical significance (unpaired t-test; see **Table S1**): *: $p<0.05$, not significant (n.s.)

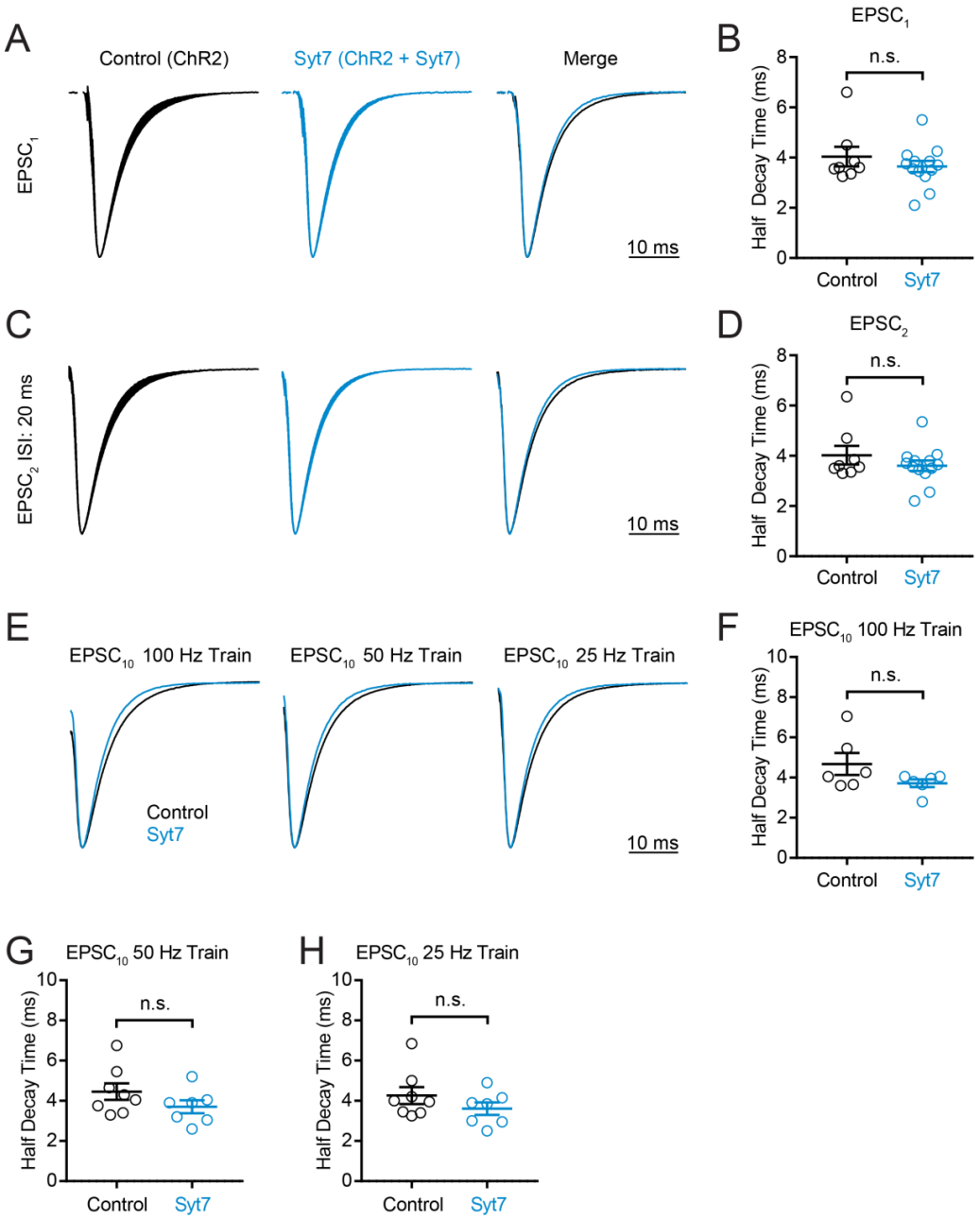

Figure S4: Synaptotagmin 7 expression in climbing fibers does not significantly change EPSC decay in low external calcium. Related to Figure 3.
Experiments were performed in ChR2 (black) and Syt7-ChR2 (light blue) injected animals at the CF-PC synapse under conditions of low (~0.3 mM) external calcium.
(A) Normalized to the $EPSC_1$ of low calcium PPR experiments displayed as average ± SEM (*left, middle*) or average only (*right*).
(C) Same as in (A) except normalized to the second EPSC of 20 ms ISI PPR experiments
(E) Normalized to $EPSC_{10}$ of low calcium train experiments. Only averages are shown for ChR2 (black) and ChR2-Syt7 (light blue) for 100 Hz (left), 50 Hz (middle) and 25 Hz (right) trains
(B, D, F, G, H) Half Decay Times are plotted with mean ± SEM indicated. Statistical significance (unpaired t-test; see Table S1) Statistical significance (unpaired t-test; see Table S1): not significant (n.s.)

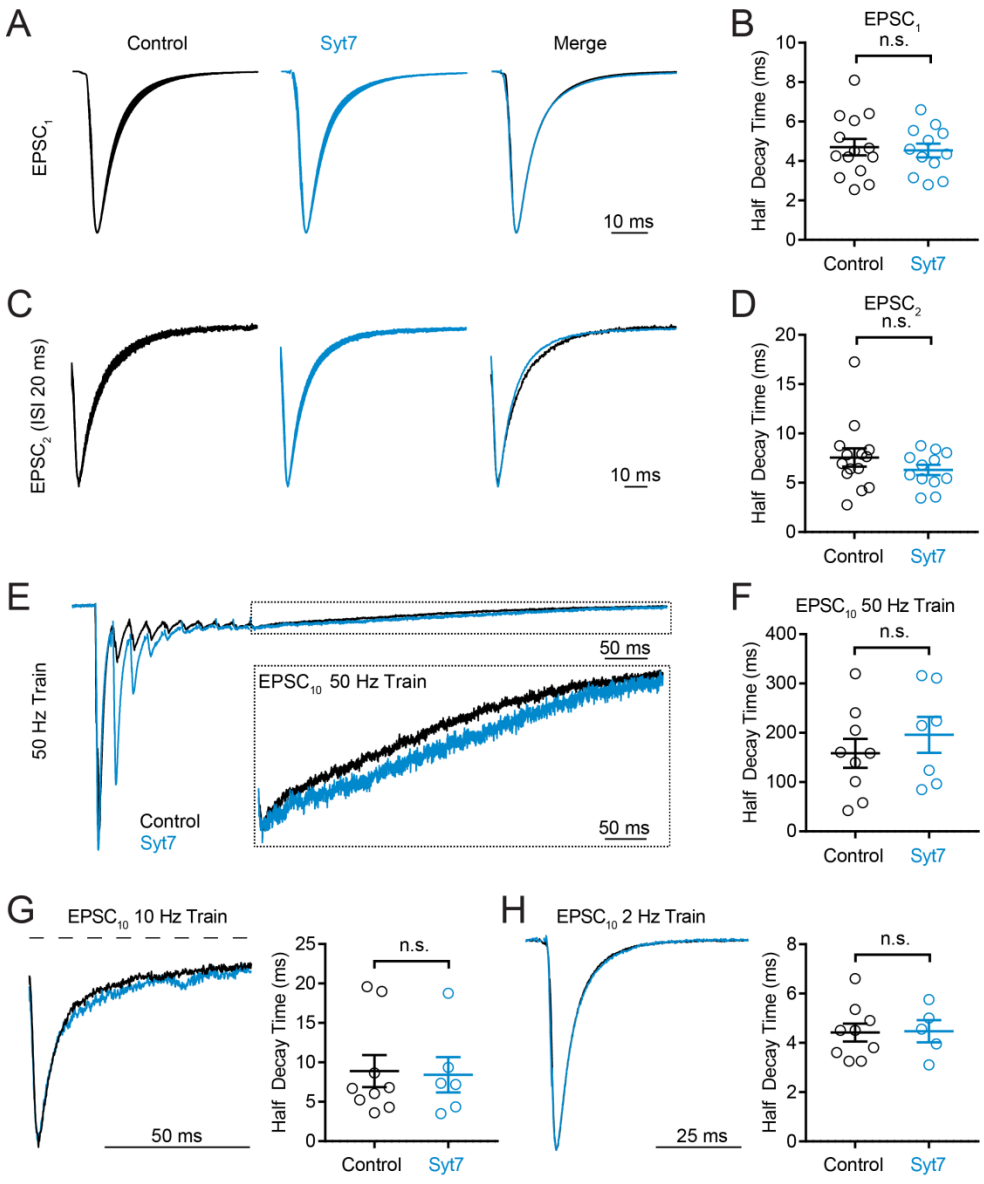

Figure S5: Synaptotagmin 7 expression in climbing fibers does not significantly change EPSC decay in high external calcium. Related to Figure 4.
Experiments were performed in ChR2 (black) and Syt7-ChR2 (light blue) injected animals at the CF-PC synapse under conditions of high (~4 mM) external calcium.
(A) Normalized to the $EPSC_1$ of high calcium PPR experiments displayed as average ± SEM (*left, middle*) or average only (*right*).
(C) Same as in (A) except normalized to the second EPSC of 20 ms ISI PPR experiments
(E, G *left*, H *left*) Normalized to $EPSC_{10}$ of high calcium 10 stimuli train experiments. Only averages are shown for ChR2 (black) and ChR2-Syt7 (light blue) for 50 Hz (E), 10 Hz (G *left*) and 2 Hz (H *left*) trains. Dashed line indicates baseline at zero (G *left*).
(B, D, F, G *right*, H *right*) Half decay times are plotted with mean ± SEM indicated. Statistical significance (unpaired t-test; see Table S1) Statistical significance (unpaired t-test; see Table S1): not significant (n.s.)

Fig.	Measurement	Genotype	n/N	Mean ± SEM	Statistical test	p
1A	PPR, ISI: 10 ms	WT	6/2	1.54 ± 0.08	Student's t-test	0.62
		Syt7KO	13/9	1.6 ± 0.07		
1B	50 Hz: $EPSC_{10}/EPSC_1$	WT	9/2	2.77 ± 0.28	Student's t-test	0.89
		Syt7KO	9/2	2.72 ± 0.28		
1C	PPR, ISI: 10 ms	WT	4/2	0.17 ± 0.006	Student's t-test	0.22
		Syt7KO	4/2	0.18 ± 0.009		
3A	PPR, ISI: 10 ms	ChR2	8/7	1.58 ± 0.07	Student's t-test	0.016 *
		ChR2-Syt7	12/9	2.43 ± 0.25		
3B	50 Hz: $EPSC_{10}/EPSC_1$	ChR2	8/7	3.62 ± 0.23	Student's t-test	<0.01 **
		ChR2-Syt7	7/5	11.3 ± 2.6		
4A	PPR, ISI: 10 ms	ChR2	13/9	0.16 ± 0.01	Student's t-test	<0.01 **
		ChR2-Syt7	11/6	0.66 ± 0.05		
4A	PPR, ISI: 500 ms	ChR2	13/9	0.76 ± 0.03	Student's t-test	<0.01 **
		ChR2-Syt7	11/7	1.14 ± 0.04		
4B	50 Hz: $EPSC_{10}/EPSC_1$	ChR2	9/6	0.03 ± 0.005	Student's t-test	0.63
		ChR2-Syt7	6/4	0.02 ± 0.006		
4C	10 Hz: $EPSC_{10}/EPSC_1$	ChR2	9/6	0.19 ± 0.03	Student's t-test	0.74
		ChR2-Syt7	6/4	0.17 ± 0.02		
4D	2 Hz: $EPSC_{10}/EPSC_1$	ChR2	9/6	0.69 ± 0.02	Student's t-test	<0.01 **
		ChR2-Syt7	5/3	0.82 ± 0.03		
4G	50 Hz: $EPSC_{11}/EPSC_1$	ChR2	7/5	0.66 ± 0.08	Student's t-test	0.87
		ChR2-Syt7	6/4	0.68 ± 0.08		
S1	100 Hz: $EPSC_{10}/EPSC_1$	WT	7/2	2.76 ±0.37	Student's t-test	0.93
		Syt7KO	8/2	2.8 ± 0.31		
S1	25 Hz: $EPSC_{10}/EPSC_1$	WT	8/2	2.21 ± 0.21	Student's t-test	0.94
		Syt7KO	8/2	2.23 ± 0.24		
S2	100 Hz: $EPSC_{10}/EPSC_1$	ChR2	6/5	3.94 ± 0.56	Student's t-test	<0.01 **
		ChR2-Syt7	6/4	10.34 ± 1.83		
S2	25 Hz: $EPSC_{10}/EPSC_1$	ChR2	8/7	3.05 ± 0.24	Student's t-test	<0.01 **
		ChR2-Syt7	7/5	9.81 ± 2.32		
S3A	EPSC1 Size	ChR2	8/7	1015 ± 147 pA	Student's t-test	0.043 *
		ChR2-Syt7	13/9	612 ± 114 pA		
S3B	EPSC1 Size	ChR2	14/9	1042 ± 114 pA	Student's t-test	0.11
		ChR2-Syt7	12/7	812 ± 66 pA		
S4B	$EPSC_1$ ½ Decay Time	ChR2	8/7	4.04 ± 0.39 ms	Student's t-test	0.36
		ChR2-Syt7	13/9	3.65 ± 0.23 ms		
S4D	$EPSC_2$ ½ Decay Time 20 ms ISI	ChR2	8/7	4.03 ± 0.37 ms	Student's t-test	0.29
		ChR2-Syt7	13/9	3.6 ± 0.21 ms		
S4F	$EPSC_{10}$ ½ Decay Time 50 Hz	ChR2	6/5	4.68 ± 0.55 ms	Student's t-test	0.13

		ChR2-Syt7	6/4	3.72 ± 0.19 ms		
S4G	$EPSC_{10}$ ½ Decay Time 10 Hz	ChR2	8/7	4.46 ± 0.41 ms	Student's t-test	0.18
		ChR2-Syt7	7/5	3.70 ± 0.32 ms		
S4H	$EPSC_{10}$ ½ Decay Time 2 Hz	ChR2	8/7	4.26 ± 0.42 ms	Student's t-test	0.24
		ChR2-Syt7	7/5	3.61 ± 0.31 ms		
S5B	$EPSC_1$ ½ Decay Time	ChR2	14/9	4.70 ± 0.42 ms	Student's t-test	0.76
		ChR2-Syt7	12/7	4.53 ± 0.35 ms		
S5D	$EPSC_2$ ½ Decay Time 20 ms ISI	ChR2	14/9	7.55 ± 0.93 ms	Student's t-test	0.27
		ChR2-Syt7	12/7	6.30 ± 0.52 ms		
S5F	$EPSC_{10}$ ½ Decay Time 50 Hz	ChR2	9/6	159 ± 29 ms	Student's t-test	0.43
		ChR2-Syt7	7/4	196 ± 37 ms		
S5G	$EPSC_{10}$ ½ Decay Time 10 Hz	ChR2	9/6	8.9 ± 2.0 ms	Student's t-test	0.88
		ChR2-Syt7	6/4	8.4 ± 2.2 ms		
S5H	$EPSC_{10}$ ½ Decay Time 2 Hz	ChR2	9/6	4.42 ± 0.37 ms	Student's t-test	0.93
		ChR2-Syt7	5/3	4.47 ± 0.45 ms		

Table S1: Statistical Tests of main and supplemental figures. Related to Figures 1, 3, 4, S1, S2, S3, S4, S5). N indicates number of animals, n indicates number of neurons

Fit: $y_0 + Aexp(-(x-x_0)/\tau)$	y_0	x_0 (stimuli)	A	τ (stimuli)
Fig.1B, WT	3.5	1	-2.4	7.2
Fig.1B, Syt7KO	3	1	-1.9	4.2
Fig.S1, 100Hz, WT	3.1	1	-2	3.9
Fig.S1, 100Hz, Syt7KO	3	1	-1.9	3.6
Fig.S1, 25Hz, WT	2.5	1	-1.4	4.9
Fig.S1, 25Hz, Syt7KO	2.4	1	-1.4	4.3
Fig.3B, Control	4.9	1	-3.9	7.6
Fig.3B, Syt7	18	1	-17	8.6
Fig.S2, 100Hz, Control	4.6	1	-3.6	5
Fig.S2, 100Hz, Syt7	16	1	-16	9
Fig.S2, 25Hz, Control	3.9	1	-2.9	7.1
Fig.S2, 25Hz, Syt7	20	1	-19	13

Fit: $y_0 + A_1exp(-(x-x_0)/\tau_1) + A_2exp(-(x-x_0)/\tau_2)$	y_0	x_0 (ms)	A_1	τ_1 (ms)	A_2	τ_2 (ms)
Fig.1A, WT	1	10	0.22	8.0	0.32	311
Fig.1A, Syt7KO	1	10	0.18	8.0	0.42	214
Fig.1C, WT	1	10	-0.39	113	-0.44	2570
Fig.1C, Syt7KO	1	10	-0.35	147	-0.47	2360
Fig.3A, Control	1.1	10	0.19	7.7	0.29	138
Fig.3A, Syt7	1.1	10	0.72	39	0.58	266

Table S2: Fit parameters. Related to Figures 1, 3, S1, S2. N indicates number of animals, n indicates number of neurons.